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ON THE HABITS AND REACTIONS OF SAGARTIA DAVISI.

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Sagartia davisi[1] is the Pacific representative of the *S. luciæ* of the Atlantic coast of the United States. The two species appear to differ only in the absence, in *S. davisi*, of the narrow yellow stripes on the column which are so characteristic of the eastern form. Both species commonly reproduce by fission, and it is probable that the following descriptions of some of the other habits of *S. davisi* may be applicable to *S. luciæ* also.

S. davisi was first discovered clustered on the valves of a species of the bivalve *Chione*, common in the harbor of San Pedro, Cal.[2] The clam dwells near or on the surface of the sand. As it plows its way along, the uppermost regions of the shell are about the hinge and the siphons. To these regions, invariably, the polyps cling. The deeper the clam goes the more do their small, thin-walled bodies lengthen, supported by the surrounding sand, to a degree I have never seen in an erect and unsupported form. *S. davisi* is not, however, a burrower, nor is the association with *Chione* a case of commensalism, as might be concluded at first sight. It fastens readily to any object which can give it a foothold and keep it out of the sand. On the sand flats at San Pedro

[1] Description. Column of largest polyps about 1 cm. in diameter; spread of tentacles about 2 cm. Foot disk very extensible; body wall everywhere quite thin and semitransparent; a distinct capitulum above a well-defined collar as in *Metridium;* oral disk almost circular, mouth small, oval, lips with about twelve lobes, not prominent; one or two, occasionally three siphonoglyphs. Tentacles tapering, slender, pointed, variable in number, most often 45–50 in perfect individuals. Color of column dark brown, tentacles and disk green. Many individuals with light longitudinal stripe of variable width on column (zone of regeneration after fission). Mesenteries unusually variable in number and arrangement. Reproduction sexual and non-sexual; latter the cause of irregularities in number and arrangement of tentacles and mesenteries.

[2] It has since been found in San Diego Bay, Cal.

it was uncovered about half each day by the falling of the tide, during which periods its tentacles were completely retracted, contrary to the custom of such typical sand dwellers as *Harenactis attenuata*,[1] and another species of *Sagartia* from San Pedro as yet undescribed. It may live permanently submerged, however, and thrives in aquaria.

Though ordinarily attached to a solid substratum, it is occasionally found free on the sand. Its powers of locomotion are considerable. By means of multicellular amœboid processes of the foot disk, readily seen with a hand lens at the edge of the disk, it is capable of creeping more than an inch in an hour. The polyps often leave the clam to which they are attached when placed in an aquarium, especially when they are on the lower valve. They may occasionally creep along the surface of the water, hanging from the surface film.

The inverted position is not long retained, however, for *S. davisi* has a marked tendency to assume as erect a posture as its situation will permit. An example of this tendency is provided in the case just mentioned, of the relatively greater haste of the polyps in leaving the lower than the upper valve of a clam lying on the aquarium floor. Moreover, the axes of polyps clinging to the vertical sides of the aquarium are either perpendicular to the sides or bent upward. They never bend downward if the polyps are submerged.

The orientation of *S. davisi* is, then, partly a result of geotropic stimulation. The same may be said of the locomotion of the species. There is a definite tendency of the polyps on the walls of the aquaria to collect near the surface, although the aquaria may be sealed jars completely filled with water, or furnished with green water plants evenly distributed (precautions against the possible influence of oxygen at the surface). The polyps on the floor of the aquarium, if it be horizontal, move about but little, and when they do, sporadically and without certainty of direction. When by chance, however, they reach the angle made by the floor and a side of the aquarium and begin an ascent, there is never a retrograde movement, seldom a halt, until they draw near the surface. This locomotor geotropism is especially inter-

[1] Torrey, 1901.

esting from the fact that the major axis of the animal is not parallel with the direction of locomotion, a peculiarity which distinguishes it from the reactions of the majority of animals to directive stimuli. The major axis is the axis of geotropic orientation, but it can only be the axis of locomotion in swimming forms[1] and those which lack a foot disk and creep on the column (*e. g., Peachia*).

It is possible to reconcile these different cases if we think of the foot disk merely as a differentiated portion of the body wall. *Edwardsia* has no well defined foot, though its aboral end is rounded and adhesive. The hydroid, *Corymorpha*, again, has no foot such as is possessed by *Hydra*, its aboral end coming to a point; yet the sides of this tapering extremity are adherent, and through their amœboid cells the hydroid, orienting negatively to gravity, tends also to move vertically upward. It adheres in this case by a portion of what may truly be called its lateral wall; in consequence of which the axes of locomotion and geotropic orientation coincide. *S. davisi* is an extreme case in the other direction. Having a large and well defined foot, it can hardly be said to cling obviously by a portion of the lateral (*i. e.*, column) wall. At the same time, when on a vertical surface, its axes of locomotion and orientation are as nearly parallel as the differentiation of foot and column will allow. This, however, is not equivalent to saying that the direction of locomotion in response to a directive stimulus is determined by the orientation of the major axis of the polyp, for the elements of the foot may be directly affected by gravity.

Loeb ('91, p. 70) has said that *Cerianthus* and *Actinia equina* went from smooth glass to a mussel shell or piece of ulva more readily than in the reverse direction in his experiments. This indicates a certain "contact irritability," which seems to be possessed also by *S. davisi*, as the latter moves about more freely on smooth glass than on rough surfaces. The reaction to the contact stimulus, however, is not so strong as the orienting re-

[1] Besides the pelagic species which Andres describes among the *Minyadidæ*, all non-adherent, there is an interesting polyp abundant in the harbor of Honolulu which, I am told by my friend Mr. Loye H. Miller, leads both a sedentary and a free existence. It appears to have no pedal float, sustaining itself by means of rhythmic movements of the tentacles which send it along foot foremost at a fair rate of speed.

action in response to the stimulus of gravity, so that as a resultant of the opposing responses, the polyp leaves the shell for the glass as stated above.

Light does not appear to stimulate *S. davisi* in any way. The polyps neither bend nor move toward the light when it comes from but one side of the aquarium, in all degrees up to the intensity of bright daylight. Neither do flashes of sunlight falling upon polyps in a darkened aquarium produce any muscular responses. *S. davisi* differs in this respect from *Cerianthus membranaceus* and *Edwardsia lucifuga*, according to Nagel ('94, p. 545).

The responses of anemones to mechanical and chemical stimuli have been investigated already by Pollock ('82), Loeb ('91 and '95), Nagel ('92, '94*a*, '94*b*) and Parker ('96). With most of the conclusions of these investigators my own observations accord. I must differ with some, adding also a few facts which to my knowledge, have not been published heretofore.

Two quite distinct reactions usually follow the stimulation of a tentacle of *S. davisi* by means of a slight touch with a needle or glass rod. The first is a bend at and toward the point of stimulation, whether the latter be near tip or base, on right side or left, above or below, and appears to be due to the response of the muscles involved to a direct stimulus. The second is a contraction of the whole tentacle, with a simultaneous bending of the tentacle toward the mouth. Evidently all the longitudinal muscles of the tentacle not previously active are indirectly excited to produce this reaction, those on the inner (upper) side between base and point stimulated contracting more strongly than the outer (lower) muscles. This unequal contraction is probably to be explained by the greater strength of the inner muscles, which play the greater part in the chief work of the tentacles — carrying food to the mouth. The hydroid *Corymorpha* shows this inequality still more strikingly; the first reaction of *S. davisi* is entirely wanting and the outer muscles are in use only when the tentacles are slowly returning to their expanded condition after a contraction. But I have been unable to demonstrate histologically any difference in size between outer and inner muscles, in either animal.

The result of the second reaction is varied. Often the tentacle merely waves stiffly inward. At other times it may arch so that its point is directed toward the mouth. On the whole, however, its movements are less definitely adaptive than those which Parker describes for *Metridium*.

The second reaction does not always follow the first. The general contraction does not appear to be induced by contact alone. If the tentacle be touched lightly and for but an instant, only the first reaction occurs. If, however, the stimulating object rest against the tentacle sufficiently long to allow the latter to adhere to it, the second reaction immediately follows. Whether this results from the adhesion itself, or the duration of the stimulus, or a tension in the muscles due to the resistance of the stimulating object, I am unable fully to decide. Such small objects are capable of producing this reaction that the third possibility seems to be excluded. Whichever of the other two be the efficient stimulus, it produces a strong contraction of the muscles directly affected. This strong contraction probably serves as a direct stimulus for contiguous muscles, the contraction of these for others, and so on, until all are involved. In no case did the evidence enforce the assumption of the presence of nerves, in the tentacular responses.

So far the movements of but a single tentacle have been considered, without relation to the others. And it should be said here that tentacles cut from the polyp behave in all essential respects as they do under normal circumstances. Often the stimulus applied to one tentacle is sufficient, unless care be used, to induce contractions in several. It may be that only a few tentacles on each side of the one touched will react; with a stronger stimulus the entire set of tentacles may contract with vigor. There is no more evidence, however, that this correlation of parts is attained by the aid of nervous tissue than there was in the case of the single tentacles. Communication from one tentacle to the next is largely through the oral disk. The proper degree of contraction of a tentacle induces a contraction in neighboring muscles in the oral disk, and possibly in contiguous tentacles directly. The vigor of the stimulus, if it be local, appears to determine the extent of the

response, which spreads by the direct effect of the tension of one muscle on those near it.

The usual response of a tentacle stimulated indirectly in the manner just described is a waving or arching toward the mouth, with or without vigorous shortening of the whole tentacle. Occasionally the response is quite opposite to this, the stimulation of one tentacle producing an outward waving of neighboring tentacles. The anomaly is only apparent, not real, for as a matter of fact the muscles of the neighboring tentacles are not involved at all in the latter case. The tentacles neither shorten nor bend. They move outward stiffly, owing to a local contraction of muscles in the oral disk or the capitulum.

If the stimulus applied to a tentacle be sufficiently strong, all of the tentacles may shorten simultaneously, may even be entirely withdrawn into the body by the contraction of mesenterial muscles and hidden by the contraction of the sphincter; the column may shorten also, and the foot disk may change its shape. All of these movements seem to be induced by the direct passage of the stimulus from muscle to muscle without the aid of nerve tissue.

The oral disk, between tentacles and mouth, is almost insensible to mechanical stimuli.

Stimuli applied to the column produce the inward movement of several or all tentacles, the outward movement of a few, or the contraction of column and foot disk, according to the strength of the stimulus. Stimulation of the foot disk, either at the edge or on the lower surface, produces local contraction of the foot and base of the column, and acontia are usually emitted near the point stimulated. The tentacles may contract also, but always as a whole, the same general reaction following stimulation at different points of the disk instead of a local reaction as in the cases of the foot, column and acontia. This inability of the tentacles to recognize the direction of the stimulus is also characteristic of the reaction of the tentacles of *Corymorpha* to stimulation of the column, and is due, I believe, to the opportunities for diffusion of the stimulation impulse owing to the distance of the reacting structures from the point at which the stimulus is applied.

The entire surface of *S. davisi*, with the possible exception of

a small zone between mouth and tentacles, responds to a mechanical stimulation, the greatest irritability being manifested by the tentacles, the tactile organs *par excellence*. The latter exhibit a very definite adaptive reaction. The preliminary bend of an irritated tentacle at and toward the point stimulated makes it possible for the polyp in a sense to pursue its prey actively if, indeed, to but a limited extent. The great advantage of this reaction over the simple inward movement of the tentacle indirectly stimulated is obvious. The latter is also adaptive, however, since it is the most likely movement to clutch food organisms in a polyp whose tentacles are habitually outstretched. Supporting this idea is the fact that the tentacles of hydroids react only in this way, whether stimulated directly or indirectly. It is the simpler, more primitive reaction.

A more efficient adaptive reaction, also indirectly induced, is the extrusion of acontia at the point stimulated, for purposes of defense. Though the reaction is always the same, the acontia always move in the most desirable direction, which is not always the case with tentacles.

The passive outward movement of tentacles due to the contraction of muscles in the oral disk or capitulum is not directed by the position of the stimulating object. It may be toward the latter, but only when the stimulus happens to be applied at a point external to the tentacles, that is, at some point which is less likely to be stimulated by a food organism than points on their inner surface. When a tentacle of an outer whorl is moved passively as a result of the stimulation of a tentacle of an inner whorl, the movement is away from, not toward the tentacle stimulated. The reaction in this direction is of no obvious importance to the polyp in this case, and seems to be of no more importance, in the sum of all cases, than a movement in any direction. It appears, therefore, to have no adaptive value whatever.

By means of its varying sensitiveness to different chemical substances and its ability to discriminate between mechanical and chemical stimuli, *S. davisi* is enabled to make certain choices in its quest for food. This capacity, which it possesses in common with other anemones, has been described as olfactory by Romanes

and Nagel, and as gustatory by Jourdain. Such expressions, however, are essentially psychological, and Loeb ('91) has justly insisted upon the substitution for them of some physiological expression, such as *chemical irritability*. This power of discrimination has been shown by Loeb to reside not only in the tentacles (Nagel, '92), but also in other regions. *Actinia equina* discriminated between crab's flesh and small rolls of paper as definitely after he had removed the tentacles by a transverse cut as before. Parker ('96) demonstrated later that *Metridium dianthus* reacts in different ways to mechanical and chemical stimuli.

Actinia equina, from Loeb's observations, is so definite in its choices that chemically inert paper pellets were never taken into the mouth. Parker found that *Metridium* would swallow pieces of white india rubber as well as flesh, though the former were sometimes disgorged before they had passed out of the œsophagus. Since he has shown that the cilia covering the lips of *Metridium* and beating outward in the absence of chemical stimuli, reverse their dominant beat in response to the stimulation of meat juices, their behavior when stimulated by apparently chemically inert india rubber leaves a doubt as to whether or not they can be reversed by purely mechanical means. There is, however, no doubt of such a reaction in *S. davisi*, as will be shown in the course of the following account of my experiments.

It may be well to begin with the effects of various chemicals. Cane sugar in solutions of various strengths produced no appreciable reactions in any part of the polyps on trial. Strong picric acid and 4 per cent. formalin caused the retraction of all the tentacles, indicating stimulation of body muscles. One half per cent. hydrochloric acid caused a general contraction of tentacles. From a knowledge of the behavior of *Corymorpha*, which, though unable to detect the presence of flesh until touched by it, yet reacts strongly to strong alcohol and acetic acid, I am led to suspect that these substances irritate the polyp in the same way that they irritate one's skin, through the tactile organs merely.

Crab's muscle, bits of limpet and annelid worm were used as

stimulators with uniform results. Small amphipods were devoured with avidity. The response differed according as the stimulus was applied locally or generally. For my first experiment I placed a small piece of worm on several of the outstretched tentacles of a polyp. The tentacles immediately adhered, bending at and toward the point stimulated, as though responding to a purely mechanical stimulus, and then contracted, dragging the morsel to the mouth. For some seconds the tentacles not in contact with it remained motionless. Then, one or two at a time, they waved slowly inward and grasped the flesh, almost every tentacle finally becoming thus engaged. This experiment was tried many times with similar results. Apparently the tentacles not mechanically stimulated were irritated by substances in solution diffusing out of the flesh, and the reaction was as definite as it would have been if induced by a mechanical stimulus. The movement was toward the stimulus.

The possibility of an indirect stimulation of these tentacles through the oral disk from the tentacles touching the flesh was eliminated by holding a piece of worm flesh immediately above the mouth of another polyp. In a few seconds some of the tentacles began to twitch slightly, and a little later all began to wave slowly inward, toward the flesh, finally grasping it. A similar result followed numerous trials.

Next, a bit of flesh was placed on the aquarium floor, near, but not in contact with the foot disk of another polyp. Would the tentacles bend in the direction of the flesh now, or toward the mouth? This experiment, repeated a number of times, did not give uniform results. In the majority of cases the tentacles waved toward the mouth, *away from* the flesh. In the rest they moved *toward* the flesh in the most definite and unmistakable manner. Not only that; the column, in several cases, bent toward the morsel which was seized by the tentacles nearest it and dragged toward the mouth. There is no doubt here that the movements were in the direction of the stimulating object, and are thus comparable to the well-known movements of the manubria of various mendusæ toward stimulated points on the subumbrella. The proboscis of *Corymorpha* reacts similarly, as will be shown in a forthcoming paper.

Pollock ('82) observed this fact but was unable to reconcile his varying results. The reason for his failure was, I believe, that he failed to distinguish between *general* and *local* stimulation. When the meat juices of the annelid used previously were discharged gently over a polyp from a pipette, I observed that the tentacles always waved inward, without regard for the direction from which the juice was coming. This general chemical stimulation produced the same response from the tentacles that a mechanical stimulation of the foot disk provoked. In both cases then, in which the tentacles waved inward and away from the flesh, the diffusion of soluble substances from the latter was probably so rapid that the tentacles were stimulated *on all sides* so nearly at the same time that no differential of stimulation beween opposite sides of the tentacles was established, the necessary condition of a directive reaction. But why the movement toward the mouth? Because it is the primitive clutching movement already spoken of as most likely to capture food organisms, in a polyp whose tentacles are habitually outstretched. It is the simplest adaptation of the prehensile mechanism, common to hydroids as well.

The responses of the tentacles to mechanical and chemical stimuli are essentially the same. The bend is toward the stimulus when the stimulation is local, toward the mouth when it is general, whether direct or indirect.

If we turn now to the phenomena of swallowing, we shall see that the cilia of both lips and œsophagus may respond to mechanical as well as chemical stimulation by waving more strongly inward than outward. I early observed that not only were pieces of flesh occasionally rejected, but bits of shell and gravel were sometimes taken in. With the idea in mind that the size and shape of the object might affect the reaction, several substances, presumably chemically inert, were given to various polyps, in pieces varying in these respects. Pieces of very thin paper, from 1 mm. to 3 mm. square, when placed npon the tentacles, were cast off in half an hour. A piece of cork, about one fourth as large as the polyp, was likewise rejected. A much smaller piece, capable of being easily ingested, was taken into the gullet and retained for thirty minutes. A piece of paraffine of similar

size was swallowed in three minutes, and a half-cube of heavy drawing paper, of about the same size, was also swallowed, though more slowly. Tiny bits of glass were frequently swallowed.

I can say definitely that these objects were not carried in by the beat of the cilia covering the siphonoglyphs and producing an insetting current, but by cilia covering the lips and œsophagus between the siphonoglyphs and producing a current which ordinarily sets outward. It would seem, then, that chemically inert substances, if small enough to be taken easily into the mouth and thus brought into direct contact with the ciliated cells lining the œsophagus, are ingested under some conditions. Other experiments show that one of these conditions, probably the most important, is the degree of hunger of the polyp. Starving polyps were always more ready than well fed individuals to swallow chemically inert substances. Some explanation of this fact may be derived from the further fact that hungry polyps are in general unusually sensitive to both chemical and mechanical stimuli. Increased sensitiveness means increased effectiveness of a given stimulus; this is equivalent to saying that the stimulus is more intense. *S. davisi*, then, responds only to certain intensities of the same stimulus, so far as the ciliated cells of the lips and œsophagus are concerned. Under mechanical stimulation of a given intensity, the cilia do not reverse their beat; an increase in the intensity or, if you will, effectiveness of the stimulation may produce this reversal. To chemical stimuli, or to mechanical which are above a certain degree of intensity (*i. e.*, when the stimuli polyp is starving), the response is usually positive; to a weakened mechanical stimulus there is less likelihood of any response.

The positive response to mechanical stimuli is undoubtedly advantageous to the polyp. It is apparent that substances with even a very small food value must be of some importance to a starving polyp although they would not be desirable as food for a well nourished animal. For the latter they would come into the category of useless substances, which the ciliary currents on œsophagus, lips and tentacles are admirably adapted to remove.

The disgorgement of non-nutritious bodies may now be briefly considered. All harmless non-nutritious bodies, and all food stuffs from which the nutrient juices have been taken during the process of digestion, are sooner or later cast out of the mouth. The cause of the ejection is to be found in the behavior, under varying stimulation, of the œsophageal cilia. The mesenterial filaments bordering the mesenteries, and the defensive filamentous acontia, are ciliated, but probably take no part in the process, for several reasons.[1]

First, the mesenterial filaments pursue excessively meandering courses along the edges of the mesenteries, and their cilia produce many currents which are antagonistic instead of proceeding in one general direction. I have not been able to determine whether the cilia beat more strongly away from or toward the mouth. In all parts of each filament, however, they appear to beat in the same direction ; and this beat is not reversed by contact with meat or meat juices. Second, the cilia on the acontia beat always more strongly toward their free ends, and they too do not reverse their beat in the presence of meat juices. Since the acontia are attached by one end only, have a marked tendency to coil, and occupy without regularity of arrangement any position in the cœlenteron, they can hardly be concerned with the phenomena of disgorgement. It may be noted in passing, however, that when they are thrust through mouth or cinclides, their cilia, in carrying toward their tips whatever foreign particles may come in contact with them, are performing what must be in the long run an advantageous service.

Finally, the œsophagus itself, ordinarily more than half the length of the column, reaches nearer to the foot disk when the polyp contracts as it does with food substances within it. The objects taken into the cœlenteron never get far away from the lower edge of the œsophagus. Under the influence of the mesenterial and acontial cilia, they may, if small enough, rotate aimlessly about during the period of digestion and absorption. In the absence of direct stimulation, the œsophageal cilia resume

[1] The following facts concerning the behavior of the cilia on mesenterial filaments and acontia were obtained from *Metridium*, but I feel confident that the same results would have followed an investigation of *S. davisi* had the supply of material permitted.

their dominant outward beat, and are able to carry away non-stimulating objects. At the end of the period of digestion and absorption, the ingested bodies have reached their minimum of stimulating power; and now, no longer able to reverse the dominant beat of the œsophageal cilia, they are carried out by the latter just as soon as they come into their sphere of influence. Why chemically inert bodies, once swallowed, should be disgorged, may be explained, I believe, by assuming inability on the part of the œsophageal cilia to continue reversing their dominant beat in the presence of a persistent or frequently applied mechanical stimulus which was originally weakly positive. This is in entire harmony with Parker's demonstration that after repeated applications of a weak chemical stimulus to the lips of *Metridium*, there comes a time when no positive reaction results.

Peristaltic movements of the œsophagus may assist the cilia, but I have no evidence that they take more than a very subordinate part in the phenomena of swallowing or disgorgement.

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January 11, 1904.

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VARIATION IN BEES.

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In the study of evolution, there is nothing more important than the investigation of variations, since the whole doctrine rests upon the premise that organisms do vary. There was a time when it was sufficient in such an investigation to take a series of specimens and from the general looks of things postulate theories. But the world has become more critical now and demands that when a statement is made concerning some phenomenon, exact data accompany the statement. Hence, the statistical study of variation which attempts to exactly measure the variations and correlations of different organs and to set them down in figures which "cannot lie." And here we cannot allow the other proverb which says that figures will prove anything, for figures truthfully handled can only prove the truth. But there is, on the other hand, great danger that, having collected a set of measurements, we make a show of accuracy that will lead us and others astray by reason of careless or insufficient analysis. Such work is most troublesome because of its seeming exactness and the difficulty of detecting errors.

Messrs. Casteel and Phillips, in the December (1903) number of this BULLETIN, have taken up a very interesting and vitally important problem. The comparative variability of the drone and worker bees hits, in a way, at the very root of the variation question. Accordingly, while we lament with the authors the smallness of their series, it seems well worth while to consider a few points about the paper.

In the first place, we have to disagree with the statement that if the variability is "due to chance," it is "not in accordance with any law," for it is well known, and has been for years, that nothing is more bound by law or more expressible in mathematical formulæ than "chance." However, we will heartily agree with them that the "true test of the relative variability" is the "descent in numbers of individuals" in the different classes as they are removed from the mean; but we wonder greatly

why they did not apply this simple test. It is called the standard deviation and must be known to everyone who has ever done any statistical work. The phrases just quoted are taken from the discussion of the counts of the hooks on the hind wings. Let us therefore examine them by means of this confessedly better measure.

We find that for the drones we have :

Lot.	No. of Specimens.	Standard Deviation.	Probable Error.
I.	50	2.1548	0.1453
II.	100	1.5435	0.0736
III.	100	1.7716	0.0845
IV.	100	1.6486	0.0786
V.	50	2.0988	0.1416
VI.	98	1.9377	0.0934

For the workers we have :

Lot.	No. of Specimens.	Standard Deviation.	Probable Error.
I.	50	1.5223	0.1027
II.	350	1.5564	0.0397
III.	100	1.5523	0.0740

This gives an average standard deviation, or variability, for the drones of 1.8592 ± 0.1028; and for the workers of 1.5437 ± 0.0721. But we see that the difference between the averages for the two sexes is less than the difference between the two sets of drones from the same hive (I. and III.); and, considering the probable errors, neither is significant. If we omit the three small series because of their large probable errors, we see that the difference between the variabilities of the two sexes is even smaller and clearly not significant. It is also unfortunate that the work should have been passed by both the authors and still two of the nine averages be wrong. The average for lot II. of the workers is 20.99, not 21.08; and that for lot VI. of the drones is either 22.65 or 22.75, according as we do or do not include the individual with 12 hooks, but it is surely not 22.42. This was probably gotten by including this individual (although he was excluded by their argument above), and then using 100 as the total number, but for some strange reason there are only 99 of the 100 said to have been studied which are listed.

Passing over the grave error of lumping the different series of ratios (p. 27) because they seemed to be alike, when really their only claim to homogeneity is that they are of the same sex and all bees — Italians, hybrids, "peculiar strains," *et al.*, from central Ohio to eastern Pennsylvania being jumbled together — let us take up the first table (vein R) as we have done the last. We find that the standard deviation of lot I. of the workers is 1.5637- ± 0.1055, and for lot V. of the drones — "from the same hive" — is 2.2517 ± 0.1519. There is here a difference of 0.6880. The probable error of this difference is ± 0.1849. The standard deviation of lot I. of drones is 2.4023 ± 0.1620 and that of lot III., of the same sex, also from the same hive is, 2.9598 ± 0.1412. The difference here is 0.5575 ± 0.2149. This is due to the extremely small size of the series measured. Since the formula for the probable error of the standard deviation is 0.6745 (stand. dev./$\sqrt{2n}$), we see how rapidly an increase of "n" — the number of individuals measured — decreases the error of the result. But it is manifest that the differences between the two sexes, as shown by these data are of no significance; for, far within the probable error of our work, we get as great a difference between two lots of drones (one lot being twice as large as either of the two considered in the comparison of the different sexes) as we get between the two sexes.

I have not taken the time to go over the rest of Messrs. Casteel and Phillip's work; but, having reached the above results with the first and the last tables will leave them to go over the intervening ones. It is also probably unnecessary to remark that, even if it turns out that the greater variability of the drones can be established, their proofs of their theory to account for this difference seem rather unsatisfactory.

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THE SEXUAL ELEMENTS OF THE GIANT SALAMANDER, CRYPTOBRANCHUS ALLEGHENIENSIS.

ALBERT M. REESE.

In the spring and early summer of 1902, the author made strenuous efforts to obtain embryological material for investigating the development of the hellbender (*Cryptobranchus Alleghcniensis*). These unsuccessful efforts have been described in an article entitled "The Habits of the Giant Salamander." [1]

In the fall of 1903, another effort was made to obtain the desired material, and a dozen or more live hellbenders were obtained as the result of a trip to the region of the Allegheny River, in western Pennsylvania.

These animals were all about the same size, 45 cm. in length, and were sent by express from their native stream to Syracuse, N. Y.

Upon opening the box, in which they were shipped, after its arrival in Syracuse, a number of eggs were found scattered through the grass that had been placed there to protect the animals during their trip. There seemed to be no difference in the coloration of the males and females, and the only way in which they could be distinguished was by the fact that, in the males, the lips of the cloaca were considerably swollen by the enlargement of an elongated mass of glandular tissue on each side.

In handling one of the ripe females of this lot of hellbenders, the author was bitten on the thumb; this was the only time in which any attempt to bite had been noticed, though many dozen animals had been handled at many different times. The bite was not at all serious, being merely a painful pinch which scarcely broke the skin. In removing one of the females from the box in which they had arrived, an egg, enclosed in its jelly-like envelope, was seen protruding from the cloaca. By gently pulling this extruded egg, it was found that a whole string of eggs could be drawn from the cloaca, without apparently injuring them in the least.

[1] *Popular Science Monthly*, May, 1903.

Each egg is a spherical yellow body, about 6 mm. in diameter, resembling somewhat the yolk of a miniature hen's egg. It is surrounded by a clear gelatinous envelope, which is arranged in two distinct layers (Fig. 1).

When removed from the gelatinous envelopes, as may easily be done by cutting through the latter with a pair of fine scissors, the egg is seen to be enclosed in a very thin and delicate vitelline membrane which is easily torn in handling.

The yolk, which is apparently evenly distributed throughout the egg, is made up of a compact mass of granules of various sizes (Fig. 2).

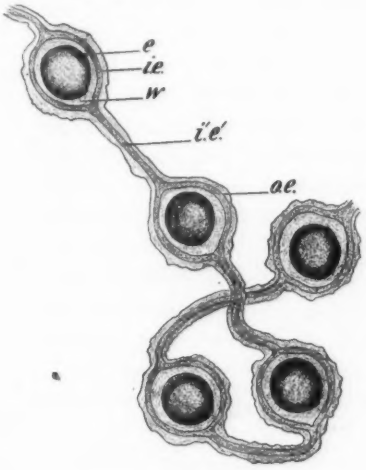

FIG. 1.

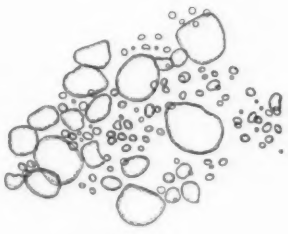

FIG. 2.

The egg is surrounded by a small amount of watery material (Fig. 1, *w*) which is, in turn, enclosed in a capsule of more dense jelly, the inner envelope (Fig. 1, *i. e.*). The inner envelope is continued as a solid, more or less tough cord of jelly (*i'. e'.*) from egg to egg, and binds them together in the continuous strings that have already been mentioned. The distance between two adjacent eggs of the string is usually about four or five times the diameter of the egg, but the elasticity of the jelly will, of course, permit the eggs to be drawn much further apart.

The outline of the inner envelope is sharp and even, while that of the outer envelope (Fig. 1, *o. e.*) is more or less irregular and uneven. The outer envelope is composed of such transparent jelly that it might easily be overlooked at the first glance. It forms a continuous layer over the entire mass of eggs.

When the unfertilized eggs are left for some days in water, they become very much swollen, by the osmosis of water through the vitelline membrane, and may eventually burst.

There was no apparent swelling of the gelatinous envelopes on coming in contact with water as is described in connection with some other amphibian eggs.

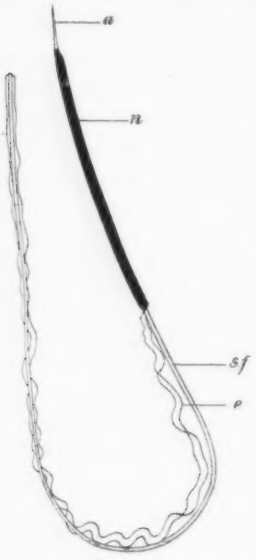

FIG. 3.

Several dozen eggs were obtained from one average-sized female, about two dozen being drawn, without apparent injury, from the cloaca, while the rest were obtained only after killing the animal and opening the body cavity. All the eggs obtained in the latter way were found to be contained in the right oviduct, the ova of the left ovary being nearly all in a very immature condition. Whether or not this was a normal condition, indicating perhaps, a very prolonged breeding season, it was not possible to say.

The spermatozoa were obtained as a milky fluid from the living males by the usual process of stripping, though considerable pressure had, in most cases, to be exerted. They were immediately examined under the higher powers of the microscope, but no motion could be detected, though it would naturally be expected that spermatozoa obtained in this way would show the usual activity of mature spermatozoa.

An attempt was made to artificially fertilize the eggs by putting them into a dish of water into which a great nnmber of spermatozoa had been stirred, but the attempt was entirely unsuccessful.

No structures resembling spermatophores were discovered, and there was nothing that would seem to give any indication of the method by which the act of fertilization was accomplished.

A single spermatozoön, as seen under a magnification of about 1,300 diameters, is shown in Fig. 3.

Fairly good preparations were easily made by drying them rapidly on the slide, and staining in hæmatoxylin and eosin.

The nucleus, *n*, is very much elongated, so that it makes up almost one third of the entire length of the spermatozoön. It is capped, at its anterior end, by a sharp, gradually-tapering apical body, *a*, which is plainly differentiated from the nucleus proper by the fact that it does not take up the stain to any great extent. No structural details in the nucleus or apical body can be discerned with the magnification used, nor is any middle-piece distinguishable. The tail, which is comparatively stout, consists of a central supporting fiber, *s. f.*, which takes up the stain slightly, surrounded by a transparent envelope, *e*, which does not stain at all. The envelope is usually considerably wrinkled and twisted, probably by the rough method of fixation.

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THE RHYTHM OF IMMUNITY AND SUSCEPTIBILITY OF FERTILIZED SEA-URCHIN EGGS TO ETHER, TO HCl, AND TO SOME SALTS.

E. G. SPAULDING.

INTRODUCTION.

The experiments described in this paper were undertaken during the summer of 1902 at the Marine Biological Laboratory upon the suggestion of Dr. A. P. Mathews; their publication has been delayed because of the pressure of other work and of the desire to, if possible, get beyond their mere description to their meaning. This end is believed to have been attained in connection with the working out of a synthesis of the artificial parthenogenetic methods, the detailed results of which attempt appear in a preceding paper.[1] The effectiveness of all these methods and so the special physico-chemical result of normal fertilization and the nature of cleavage processes can, it is believed, be explained from a unitary standpoint, viz.: if it is considered that in the process of cleavage an average decrease in surface tension takes place as a result of the equilibrating of a potential difference between osmotic pressure and surface tension, accompanied by such electrolytic changes as cause the constricted form. That this average decrease in surface tension takes place is a necessary inference from the change from the approximately spherical to the constricted form of the egg at cleavage, for this means an increase in surface. It carries with it, therefore, the decrease in that potential, osmotic, which opposes surface tension in direction. The preceding cause in these events is the creation of a potential difference by first increasing the osmotic pressure, which is done artificially by each of the parthenogenetic methods. Accordingly with the equalization of this difference, caused, *e. g.*, by a splitting up of colloidal particles or molecules, there is in the case of eggs of marine forms an absorption of water. Both of these

[1] Spaulding, E. G. "The special physics of segmentation as shown by the synthesis, from the standpoint of universally valid dynamic principles, of all the artificial parthenogenetic methods." BIOLOGICAL BULLETIN, February, 1904.

last events either alone or together may constitute what is termed liquefaction.

The results of the experiments given below can, it is believed, be interpreted in agreement with this view of segmentation. They were undertaken primarily as an extension of experimental work which had already been done on the action of various chemical compounds on protoplasmic bodies, but were limited to the study of such action on the eggs of *Arbacia* at successive periods after fertilization.

The existence of a rhythm of immunity and susceptibility has been shown already by Lyon in studying the effect of KCN and of lack of oxygen upon the fertilized eggs and embryos of the same form,[1] and it has been found also that many eggs do not segment at all in the absence of oxygen, notably *Arbacia*[2] and *Ctenolabrus*.[3] Lyon also found this summer that *Arbacia* eggs required more oxygen during precleavage and gave off more CO_2 during cleavage than at other times.[5]

From these results it may be inferred in analogy to a large number of instances well known in chemistry that at least the *ultimate* effect of oxygen on the processes conditioning cleavage is the causing of analytic chemical changes; *i. e.*, fermentation, one might say, occurs and CO_2 is given off, as Lyon found. Previous to this, however, synthetic processes may take place which in turn as certain preferments become active[4] give rise to molecular splitting. The result of such analytic change is that increase in osmotic pressure and therefore the creation of that potential difference between it and the surface tension which we have found to be necessary and in the equalization of which both potentials decrease, water is absorbed, and the egg cleaves.

The hypothesis to be deduced from this and which might serve as a guide in our experimentation is that any method either (1) of preventing this necessary preliminary increase in osmotic pressure, or of compensating it after it has been created, or (2) of increasing it beyond a certain point, will tend to do away with

[1] Lyon, E. P., *American Journal of Physiology*, VII., 1.

[2] Lyon, *loc. cit.*

[3] Loeb, J., *Archiv für die gesammte Physiologie*, 1895, LXII.

[4] Cf. Hofmeister, "Chemische Organisation der Zelle."

[5] Personal communication.

the event of cleavage. To the first two possibilities correspond the effect respectively of lack of oxygen and the use of strongly hypertonic solutions on the fertilized egg; to the second method our own results with ether, HCl, etc. From this hypothesis it can also be reasonably inferred that the nearer to the point of termination of the preparatory process that either method is used, so much the less will its effect in general be, and this supposition is again confirmed by experimental results.

Lyon[1] found that the effect of KCN on the fertilized *Arbacia* egg, taking, *e. g.*, various strengths of a titrated solution mixed with sea water, was the indication of "successive stages of relatively high and low resistance in each cleavage." Putting the eggs into the solution at successive five-minute periods after fertilization and allowing them to remain perhaps one hour, then washing and removing to sea water, he found that "there is a stage about ten or fifteen minutes after fertilization when the egg is especially susceptible to KNC." "Again *soon after* the first cleavage comes a second stage of small resistance; a third follows the second division." "The resistance of the egg to KNC increases up to a *maximum* up to the time of *separation into* the two cells." "The effect of KNC is the same as lack of oxygen."

In interpretation of these results Lyon says that the processes dependent upon oxygen seem to begin about 10–15 minutes after fertilization, for if they are inhibited the egg does not segment and they recur at each segmentation. To identify them with the morphological processes of the splitting and separation of the chromosomes or with the dissolution of the nuclear membrane seems to him to be impossible, for these occur too late to be directly affected. Wilson and Matthews,[2] he says, mention however two processes which occur sufficiently near to the susceptible stage to be worthy of consideration in this respect. One is the growth and division of the sperm aster, the other the growth of the nucleus. From the part which in order to explain the constricted form of cleavage[3] must be attributed to, as played by each of these processes, the supposition that they are affected by a lack of oxygen, by KCN, etc., receives confirmatory evidence.

[1] *Loc. cit.*

[2] *Journal of Morphology*, 1895, X., p. 319.

[3] Lillie, R. S., BIOLOGICAL BULLETIN, IV., March, 1903; and *Am. Jour. of Physiology*, VIII., 4, Jan. 1903.

But furthermore, whatever the morphological elements may be, it must also be admitted that in the processes leading up to and culminating in cleavage, we are dealing with chemical and electrolytic and consequently also with osmotic phenomena, coexisting with those of surface tension. That these first two which condition the other two are, however, not uniform, but, rather, are varying, *i. e.*, rhythmical, during that period must be admitted to explain the observed rhythm in morphological changes.

The experiments herein described serve the purpose then of testing the above-mentioned hypothesis of the existence of a liquefaction during the event of cleavage, and of a rhythm of increasing immunity up to and of marked *susceptibility during* that time. To this end use was made of ethyl ether, HCl, KCl, NaCl and sodium citrate solutions.

Theories of the Nature of their Action.

From the position that has been taken in this and a previous paper that, inasmuch as in protoplasm we are dealing with colloidal (probably also electrolytic) particles in solution, we therefore in segmentation necessarily have to do ultimately with the relations of two kinds of energy, osmotic and surface, and that the cleavage process itself depends upon the existence of an uncompensated potential difference between these, from this it follows that this potential difference might be caused *in either of two ways*, viz., at the same time that either one is kept constant, by changing the other, *i. e.*, either increasing the osmotic or decreasing the tension factor, the former being identical with the energy of the particles in solution, the latter with that of the solvent. The theories also which we find advanced in order to explain the nature of *stimulation* seems to us to be in complete agreement with this view. For example, we find the statement that "stimulation consists in the precipitation, *i. e.*, gelation, of colloidal particles and is due in the case of positively charged particles to the negative ions, *i. e.*, to the charge;[1] the inhibition of this stimulation, *i. e.*, what in some cases is termed poisoning, to the positive ions"; for negative particles the converse would hold

[1] Mathews, A. P., "The Nature of Nerve Stimulation, etc.," *Science*, March 28, 1902.

true. In any case the osmotic pressure would necessarily be affected. Both the stimulating and poisoning effect have furthermore been correlated with the valency.[1] In the case of a compound of anion and kation, both of which are monovalent, like NaCl which does and KCl which does not stimulate easily and when in both therefore the charges might seem to offset each other, the stimulating effect, *e. g.*, on the nerve has nevertheless been said to be due to the "overbalancing" of the kation by the anion, and conversely for the inhibitory effect. This of course is not real explanation unless the difference in effect can be correlated with a difference in some such quality as velocity of diffusion or solution tension, and Mathews has this summer shown that the poisoning qualities of the metals and non-metals as well are in fact a function of this latter. Some ground for this "overbalancing" effect seems to be furnished by the fact that in the case in which *e. g.*, a divalent anion is combined with a monovalent kation (2) a greater stimulating effect is observed. Thus KCl does not stimulate the nerve at its osmotic pressure, K_2SO_4 does occasionally, K_3 citrate stimulates in solutions of a gram molecule to 22,000 c.c. H_2O. But even here the number of opposing charges is the same. The kations therefore differ in some way other than in their mere number of charges. This must also hold true of the anions because of the increasing stimulating effect on the nerve of NaCl, NaBr, NaI and NaFl. The suggestion has been made that the difference in effect when the charges are the same in number is due to a difference in the translatory path of the charge around the atom; but as a cause for this latter difference must in turn be assigned the admission of an ultimate difference in the atoms themselves would seem to be necessitated.

While the salts therefore seem to affect the colloidal particles directly, the known inhibitory action of the anæsthetics would accordingly have to be identified with a direct effect on the solvent and so only indirectly on the solute. Thus it may be considered that the anæsthetics as being better solvents in most cases than water have the same effect on colloidal particles as do like charges, which repel; therefore they increase the osmotic

[1] Loeb, *Archiv für die gesch. Physiologie*, Bd. 88.

pressure. It is believed that on this basis the varying effect of ether on the eggs of *Arbacia* at successive periods after fertilization can be explained.

Experimental.

1. *The Effect of Ethyl Ether.*

The general methods of experimentation may be outlined as follows: First, a slightly supersaturated solution of ether in sea water was prepared, kept tightly corked, and when used a portion was drawn from the bottom, thus ensuring a saturated solution. The eggs were fertilized in the usual way, good lots from among a number being selected. At successive periods these eggs were transferred to staining jars containing 50 c.c. of the solution used. The strength of the solution actually used was accurately controlled by starting with twice that strength and then diluting exactly one half, in part with the sea water necessary for transferring purposes. The eggs were allowed to stand in these *covered* jars for the length of time selected; the solution was then carefully drawn off; the eggs were thoroughly washed twice with sea water, which was again added, and given time to develop. In important experiments the lots were each observed twice. All the experiments were conducted at the room temperature, about 20° C.

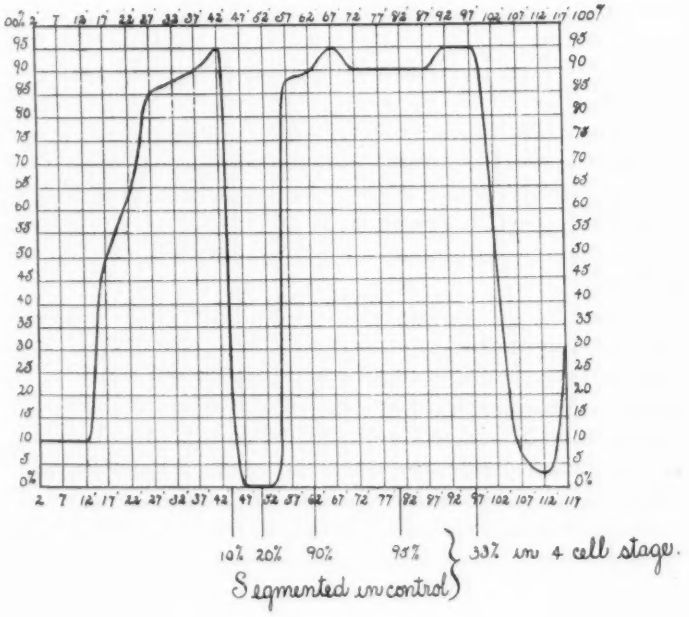
The practical problem presented was to get such a strength of solution and time of exposure that of the eggs transferred at the various periods after fertilization some would be stopped in their development, others not. For each value of the one factor there would probably be a corresponding value of the other, the product being a constant. An abstract record of what were essentially preliminary experiments is given on the next page.

From these results it seemed that the correct relation between the two variables, time and solution strength, had been found; accordingly in the next experiment a one sixty-fourth saturated ether solution was used for 25′, which gave very satisfactory results. These are tabulated and plotted on page 008.

In the plotting of the curve of these results the abscissæ represent the times of transferral after fertilization, the ordinates the per cent. of swimmers found by as accurate observation as possible.

Exper.	Strength of Solution.	Period After Fertilization.	Time of Exposure.	Observation.	Control.
I. July 24.	Saturated.	Every 15′. 8 lots.	1 hour.	All dead in the stage of treatment.	Well developed
III. July 26.	¼ and ⅛ sat. sol. by diluting with sea water (same lot of eggs).	Just before and after each cleavage.	Various.	All dead.	Well developed
IV., A. July 29.	$\frac{1}{16}$ sat. sol.; one lot of eggs for A, B, and C.	At "critical" periods, *i. e.*, just before and after each cleavage, as below in B.	30′	All dead.	Control good, regular.
IV., B.	$\frac{1}{32}$ sat sol. Lot (1).	15 minutes. (Lyon's 1st critical point.)	45′	50% unsegmented, but pigmented and swollen, some in 4 and 8 cell stage; 20% swimming.	"
	(2)	50′, 1st. cleavage just beginning.	30′	80% dead, swollen, pigmented, 20% swimming.	"
	(3)	1 hr. 10′, toward end of cleavage.	30′	87% dead, irreg. segment., some in 16 cell stage 13% swimming.	Segmentation going on.
	(4)	2 hrs., during 2d. cleavage.	30′	All stopped in 4 cell stage.	Control good.
	(5)	2 hrs. 40′, after 2d. cleavage.	30′	All dead.	
IV., C.	$\frac{1}{64}$ sat. sol. (1)	15′, Lyon's critical point.	45′	8 hrs. afterward all had segmented, 8, 16, 32 cell stage; 25% swimming.	Control good.
	(2)	50′, cleavage just beginning.	30′	(Like C 1.)	
	(3)	70′, toward end of cleavage.	30′	Nearly all swimming.	
	(4)	2 hrs., during 2d. cleavage.	30′	2–32 cell stages present; decomposed and pigmented; $\frac{1}{15}$ swimming.	
	(5)	2 hrs. 40′, after 2d. cleavage.	30′	$\frac{1}{8}$ swimming.	

Exper. V, July 30.	Solution 1/64 Sat.	Time After Fertiiiza-tion.	Exposed to Solution.	Observation.	Control.
Lot 1		2′	25′	20% swimming.	
" 2		7′		20 "	
" 3		12′		20 "	
" 4		17′		50 "	
" 5		22′		62½ "	
" 6		27′		85 "	
" 7		32′		88 "	
" 8		37′		90 "	10% in 2-cell stage, 44′ after fertilization.
" 9		42′		95 "	
" 10		47′		0 "	20% segmented.
" 11		52′		all dead, pigmented, and swollen.	20% "
" 12		57′		87.5% swimming.	
" 13		62′		90 "	90% "
" 14		67′		95 "	" "
" 15		72′		90 "	
" 16		77′		90 "	
" 17		82′		90 "	95% "
" 18		87′		90 "	
" 19		92′		95 "	
" 20		97′		95 "	33% in 4-cell stage.
" 21		102′		55 "	
" 22		107′		7.5 "	
" 23		112′		2 "	
" 24		117′		30+ "	



The character of this rather remarkable curve is obvious. Up to within twelve minutes after fertilization the resistance remains the same, but from this point on it gradually rises up to either *just before* or the *beginning* of the first cleavage; during the early part of cleavage it falls to zero, with a sharp rise afterwards and a fall at the second segmentation.

The more important question however is to get at its meaning. To get at this we take, corresponding to the general rise in immunity up to the time that cleavage is beginning, the greater demand for oxygen, established by Lyon in his work this summer. This might mean in view of the fact that either at least just preceding or *for some time* before cleavage an increase in osmotic pressure must take place, as we have shown, either one of two things to account for this, viz., either that the oxidation process is at first synthetic and subsequently determines analytic events; or that it is analytic from the start. Also to be correlated with this is the known effect of ether as a better solvent than is water. This is identical with its causing a greater degree of solution and consequently an increased osmotic pressure. The inhibiting effect of the ether on the eggs at the critical period in the above curve may be ascribed then, we believe, to its *augmentation* of the normal predominence in osmotic pressure at that time, *i. e.*, to its increase of that difference of potential in the direction of pressure-tension *necessary* for cleavage. Accordingly in the equalization of this *augmented* potential difference the eggs would be expected to increase in size more than usual in their attempt to divide, and this is confirmed by the *observed swollen* appearance, even when as in some cases division takes place once and then stops.

This increasing immunity up to the maximum can be explained then in two possible ways. If synthetic as simple oxidation processes procede the analytic then during that period there is something to *oppose* the dissolving effect of the ether; but since this *opposition* would seem to exist *equally* all through the precleavage period, the *rise* in immunity would be hard to account for in this way. On the other hand if analytic processes take place from the start as a result of the use of oxygen then the *longer before* cleavage that the exposure to ether is made the *greater* should be

its effect in augmenting the normally occurring increase in the pressure, and the point of greatest susceptibility would be at such points and also at that of the normal maximum pressure, viz., just before or during cleavage. This explanation therefore accords best with the sharp rise in the curve, and is supported also by the evidence from the parthenogenetic methods for *Arbacia*, in which the pressure is first increased, *sometime before* the cleavage, which takes place after the return from the hypertonic solution to the sea water.

The characteristics of the above curve were confirmed in general by *Experiment IV.*, B and C, already presented, and more especially by three subsequent experiments, as can be seen from the following records:

EXPERIMENT VII.

August 22, 1/64 sat. sol. Time of exposure, 1/2 hr.

Lot.	Period After Fertilization.	Observation.	Control.
1	3′	66% unsegmented.	
2	19′	90% living.	
3	31′	90% "	
4	56′	95% dead, many in 2-cell stage, pigmented, swollen.	Middle point of segmentation.
5	63′	25% swimming.	
6	79′	25% "	
7	96′	All dead in 4-cell stage.	During second cleavage.
8	112′	" "	" " "

Experiments VIII. and IX., August 23 and 29. Solution, one sixty-fourth saturated ether, one half hour exposure. Lot 1, one half hour after fertilization all living. Lot 2, during segmentation, *all dead* in 2-cell stage.

EXPERIMENTS WITH HCl.

Preliminary and theoretical. According to the views that we have previously discussed the hydrogen ion in the case of the nerve is held to inhibit the stimulating action of the chlorine ion, "overbalancing" this more than do either K, Li, NH_4 or Na. This may be due to the greater velocity of diffusion of H, which is 325, that of Cl 70.2 at 25° C.[1] On the other hand it has been supposed that the H ion brings about the parthenogenetic devel-

[1] Ostwald.

opment in *Asterias*. These two seemingly contradictory effects cannot, however, be so in reality and the difficulty may be done away with if it is borne in mind that the effect depends as much on the character (electronic) of the colloid as on the agent. For on positively charged particles the H ion would have a repelling, *i. e.*, dissolving; on negatively charged, the opposite effect.

If all the protoplasm of the *Arbacia* egg was uniformly positive just prior to or during segmentation it accordingly might be deduced that H ions would have the same effect in increasing the osmotic pressure as does ether, and so of inhibiting development. Lillie,[1] however, has shown that at cleavage the cytoplasm is markedly electropositive, the nucleus negative. Accordingly at that time he holds that the periphery repels the free kations within the egg and the nucleus the anions, so that the kations then predominate at the center, the anions at the periphery. As like charges repel each other, this is made to account for the constricted form of the egg at cleavage. In agreement with this view the effectiveness of the H ions, in parthenogenetic methods, *in the environment* might be considered to be due to their induction of a predominance of negative charges at the surface and this in turn of positive charges at the astral centers. If this be so, however, then other kations ought to have the same effect; but they do not. This indicates a specific action by the H ion, which it might have in accordance with its high diffusion velocity. It alone might therefore be considered to penetrate the egg membrane because of its and the latter's definite chemical make-up; yet there remain difficulties even here in explaining why it should do this, since, if, at least before cleavage, the membrane and cytoplasm are themselves positive they would tend to repel the H ions rather than to attract them. Only provided the surface were negative from the start could the attraction be explained. If, however, the egg, when *just about* to divide, were put into such a medium of H ions, it is reasonable to suppose that since the surface at least then is negative these might be attracted; but again it is difficult to understand how they can go further, since the cytoplasm is even yet positive. However, if they succeed

[1] Lillie, R. S., *Am. Jour. of Physiology*, VIII., IV., and BIOLOGICAL BULLETIN, IV., 4.

in acting on the cytoplasm the effect would be a repulsion of its particles and an increase in pressure, the same as that of ether. Accordingly it would *a priori* be probable that the period of least immunity to HCl would coincide with that of the greatest normal pressure, viz., just before cleavage.

Experimental.

Two preliminary experiments with seventeen different strengths showed that the proper strength of solution, for an exposure of one half hour, was between a $\frac{1}{400}$ and $\frac{1}{500}$ normal HCl soluion. Accordingly a $\frac{1}{450}$ *n* solution was next tried with the following results:

Experiment IV.

August 3, 1/450 *n* HCl solution (by diluting with sea water); three series, 25′, 30′ and 35′ exposure.

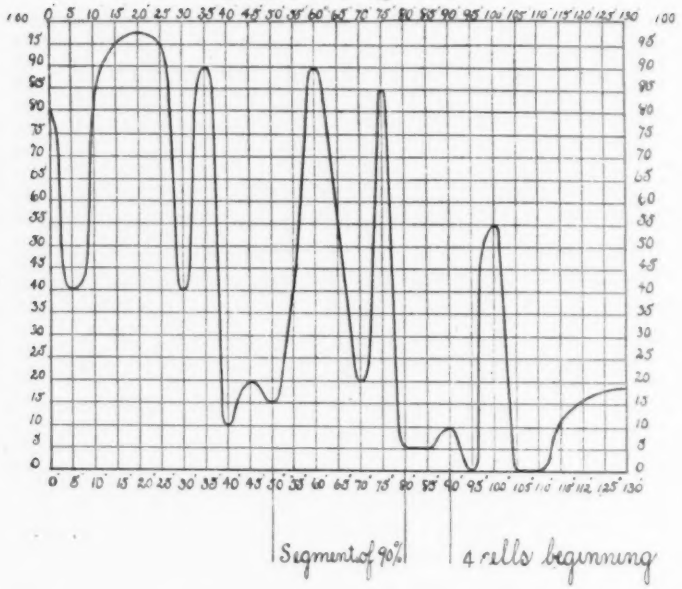
Lot.	Time After Fertilization.	Series A, 25′. Per Cent.	Series B, 30′. Per Cent.	Series C, 35′. Per Cent.	Control. Per Cent.
1	1′	No segmentation at all.[1]	A few swimmers.	A few swimmers.	
2	16′	50 swimming. 30 blastulæ, stopped.	80 swimming.	50 swimming. Gastrulæ.	
3	31′	*Quite undeveloped and disintegrated.*	Like A[3].	Like A[3].	
4	41′	80 swimming.	40 swimming.	40 swimming.	Segmentation began here.
5	46′	*All dead in 1- and 2-cell stage.*	Like A[5].	Like A[5].	Segmentation going on.
6	53′	40 swimming.	20 swimming.	20 swimming.	Toward end of cleavage.
7	59′	50 "	50 "	50 "	
8	77′	*All dead in 2- and 4-cell period.*	Like A[8].	Like A[8].	15 were in 4-cell stage.

August 5, *Experiment V.*, $\frac{1}{450}$ *n* HCl solution, used every five minutes for one half hour. The results are plotted on the next page, the times after fertilization being the abscissæ, the percentage of swimmers the ordinates, and are in general confirmatory of the previous experiments with HCl.

Two other experiments were made with HCl, which gave a general confirmation of the two described. These all agree in giving a rise in immunity soon after fertilization, then a fall at

[1] The difference between A^1, B^1, and C^1 may be due to the effect of the necessary manipulation on the eggs transferred so soon after fertilization, viz. A^1; B^1 and C^1 were necessarily transferred a little later. Cf. Mathews, *American Journal of Physiology*, VIII., IV., "Importance of Mechanical Shock on Protoplasmic Activity."

EXPERIMENT V., 1–450 *n* HCl.



30′, followed by a second rise and a second fall respectively before and during segmentation. This fall is repeated at the beginning of the second cleavage.

Comparison of this with the 'ether curve' however shows a difference in respect to this 'thirty-minute fall,' and that this should be so is to be expected from the possibility of the agent here used (HCl) directly affecting the colloidal particles as well chemically as electrically, as the evidence for a specific H ion action indeed indicates; while in the case of ether the solvent alone is first concerned.

Assuming that the H ions in some way penetrate the egg, the increase in osmotic pressure resulting therefrom might be connected with the dissolution of the nuclear membrane, which occurs at about the time of the first, the 30′ fall,[1] in such a way that an abnormal increase in pressure results from both, which inhibits development. This however could not be due alone to the electronic action of the ions for were this so ether also

[1] Mathews and Wilson, *loc. cit.*; cited by Lyon.

would have the same effect in increasing the pressure abnormally and causing a fall in immunity at the same time, which it does not do. Hence from this, together with the evidence, in other instances, of H acting differently than do other ions, and the probability that the dissolution of the nuclear membrane is caused or accompanied by chemical changes, it may be inferred that the H ions have a specific chemical effect, which, in addition to their electronic action, accounts for the fall in immunity at about 30′ after fertilization.

Both series of experiments agree, however, in making the period of greatest susceptibility just at the beginning and during the earlier part of cleavage, though for the HCl this drop seems to come somewhat earlier than for ether. The explanation for this effect of HCl we believe to be essentially the same as for that of ether, viz., that the H ions in some way penetrating the membrane cause a repulsion of at least the cytoplasmic particles, thereby *augmenting* the normally increased pressure to such an extent that further development is inhibited.

Experiments with KCl, NaCl and Na citrate.

The acceptance of a specific action for the ions is, of course, by implication not limited to H, but is quite as necessary for others, like K, Na, Ca, etc. For instance Loeb in one place holds that the kations (specific) and not the anions are poisonous,[1] for the reason that the newly fertilized *Fundulus* egg will develop in KCl but not in NaCl [2] and because, of fertilized *Arbacia* eggs in $\frac{5}{8}n$ NaCl only 10, 20, and in one case 50 per cent. began to segment, the majority stopping in the 2-cell stage, while in $\frac{5}{8}n$ KCl, 70–80 per cent. segment to 8 cells.[3] Opposed to this view of Loeb's is that which ascribes toxic effects also to the anion, for, *e. g.*, NaCl, NaBr, NaI and NaFl have a different poisoning effect. The two views, however, are quite compatible if the colloids affected are in the two cases of different sign. Loeb also finds that the toxic effects of Na salts is a function of the valency, increasing from the acetate to the citrate.[4] Further-

[1] Loeb, *Am. Jour. of Physiology*, III., VII. and VI., VI. and *Archiv für d. ges. Physiol.*, Bd. 88, 1901.

[2] Loeb, *Am. Jour. of Physiology*, III., VIII.

[3] Loeb, *Am. Jour. of Physiology*, III., IX.

[4] *Am. Jour. of Physiology*, VI., VI.

more, as an illustration of the lack of a consistent theory here, are the views of Loeb, that the antitoxic as well as toxic effect is a function of specific kations, and of others that where the kations are toxic the anions are antitoxic. For instance, in 100 c.c. $\frac{5}{8}n$ NaCl + 8 c.c. $\frac{1}{64}n$ $CaSO_4$ or $Ca(NO_3)_2$ 70 per cent. of *Fundulus* eggs develop, while if Na_2SO_4 be substituted they do not. Ca is therefore considered to be antitoxic to Na. Al_2Cl_3 and $Cr_2(SO_4)_3$ also have the same inhibitory effect on Na, but in smaller quantities. Loeb therefore concludes that the toxic and antitoxic effect of the ions is a function of their valence, but that only kations are poisonous. The necessity for such "balanced" solutions also holds good according to Loeb for muscle and for the contractions of *Gonionemus;* "margin and center must contain three ions, Na, K and Ca."[1]

The opposite view is that in the instance of, *e. g.*, NaCl poisoning but KCl not, the difference in effect is due to a greater overbalancing by the Cl ion in one case than in the other.[2] The effect is due in any case to both the ions. Both may be either toxic or antitoxic according as the colloid is like or unlike in charge and the normal event is one of gelation or of liquefaction.

Accordingly if the normal progress of cleavage in *Arbacia* demands liquefaction, *i. e.*, increased pressure and absorption of water, any salt either preventing this by unlike charges or augmenting it by like beyond a certain point may be assumed to interfere with or inhibit division. And from the data at hand we may expect NaCl to do this to a less degree than KCl.

Experiment IV., 7 pts., $\frac{5}{8}n$ KCl sol. to 2 of sea water, the other conditions as in III., gave *exactly the same* results, and is tabulated on the next page.

Examination of this record makes manifest a decrease in immunity beginning 15 minutes before cleavage, much as with HCl, but reaching its *maximum during segmentation.* The same *explanation* of this effect that was made for HCl and ether holds good, we believe, here; but the thirty-minute fall obtained with HCl is not present in these series, which indicates a different specific chemical effect of H and of K ions. Two subsequent KCl experiments confirmed these results.

[1] *Am. Jour. of Physiology*, III., VII.

[2] Mathews, *Science*, March 28, 1902, and May 8, 1903.

EXPERIMENTS WITH KCl.

	Solution.	After Fertilization.	Exposure.	Observation.	Control.
Exper. I., Aug. 16.	$\frac{1}{3}n$.				
Lots I.–III.		At *critical* periods before cleavage.	1 hour.	90–95% swimming.	Good.
Lot IV.		During cleavage, 66′ after fertilization.	1 "	75% swimming.	
Exper. II., Aug. 17.	$\frac{1}{12}n$ KCl.		1 hour.		Good.
Lots I.–IV.		Before cleavage.		All dead in 2 cells.	
Lot. V.		During cleavage.		Dead.	
Exper. III., Aug. 19.	8 pts. $\frac{1}{3}n$ KCl + 2 of sea H_2O in transferring.	every 5′	1 hour.		
Lot I.		5′		80% swimming.	80% swimming.
" II.–VIII.		(10′–40′)		80 "	
" IX.		45′		70 "	
" X.		50′		60 "	
" XI.		55′		50 "	
" XII.		60′		25 " Remainder dead and disintegrated.	Segmentation began here, 60′ after.
" XIII. and XIV.		65′–75′		5–10% swimming, remainder pigmented and disintegrated.	

Experiment I., August 29, with NaCl, 15 pts. *n* to 1 of sea water, exposure one hour, at chosen critical periods. Observation showed a slight rise in immunity from 70 to 95 per cent. up to the beginning of segmentation, then a fall to 70 per cent. during that process, but as opportunity for further experimentation was lacking this result is hardly conclusive, though by itself it is confirmatory of the supposed greater immunity to NaCl than to KCl.

Experiment I., with the Na citrate, August 13; 3 series, viz., 2 c.c. of seven-fourths *n* Na citrate to 300, 400, 500 c.c. respectively of sea water, *diluted* one half at transferral. 15′ periods, 1 hour exposure. Parallel observations showed that the eggs so treated were *ahead* of control both in beginning to segment

and in the number segmented at all cleavages. No period of susceptibility was indicated. This acceleration effect might according to the views above discussed be ascribed to the so-called "overbalancing effect" of the trivalent radical, but the experiment was not repeated because of lack of time.

Summary and Conclusions.

1. There is a pronounced *rise* in *immunity* of fertilized sea-urchin eggs to *ether* up to either *just before* or the *beginning* of segmentation; the exact point is impossible to determine owing to the unevenness of the cleavage. A *sharp decrease* then occurs, followed by a *sharp rise toward the end* of the cleavage. A repetition of this occurs at the second segmentation.

2. *Similar* changes are found resulting from the use of HCl, KCl, and NaCl with the difference that the fall in immunity comes somewhat earlier with KCl than with HCl and with this than with ether. All of these differ therefore from Lyon's interpretation of the susceptible points with KNC as occurring "after division," nor is a 15′ period found, although with HCl there is a fall in immunity at 30′ after fertilization.

3. The marked decrease in immunity "at cleavage" caused by the four agents employed seems to be explainable on the basis that all, in one way or another, *augment* beyond a certain point the increase in osmotic pressure normally necessary for cleavage. The results obtained seem therefore to be confirmatory of the position presented by the author elsewhere[1] that cleavage is due to the equalization (Ausgleichung und Umformung) of an uncompensated potential difference between osmotic pressure and surface tension, accompanied by electronic phenomena which cause constriction.

The author wishes to acknowledge his appreciation of the many opportunities offered him by the Laboratory and of the kind advice and suggestions of Dr. Mathews.

College of the City of New York,
Department of Philosophy, January, 1904.

[1] Biological Bulletin, February, 1904.

BUDDING TENTACLES OF GONIONEMUS.

GEORGE T. HARGITT.

While looking over specimens of *Gonionemus* for class work, and being somewhat on the lookout for any cases of variation, etc., my attention was arrested by an unusual appearance of a tentacle. Near the distal end was present a small knob which at first glance appeared simply as a protuberance, apparently without any very definite form or structure.

On further and more careful examination it seemed to me to warrant a careful study. It was somewhat similar to some of the conditions found by Hargitt[1] in his work on the variation of

FIG. 1.

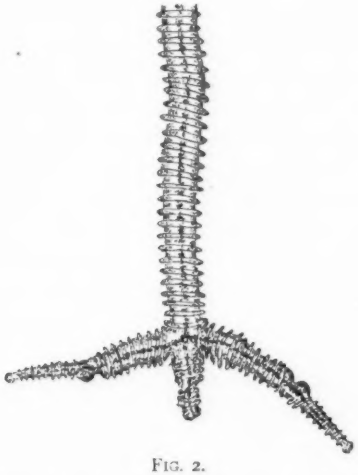


FIG. 2.

this form. The conditions referred to are the presence of bifurcated tentacles. He found a number of tentacles which had double, or in one case triple tips. In all these cases the extra tip or bud seemed to arise either from the suctorial pad or else immediately proximal to it. Fig. 1 represents a rather typical example of bifid tentacle. The specimen is one I found in C.

[1] "Variation among Hydromedusæ," BIOL. BULL., Vol. II., No. 5, 1901.

W. Hargitt's collection, but which he did not use in his paper on variation. The extra tip is seen to be much shorter than the main tip. Each tip is supplied with one of the suctorial pads, but the shorter one arises from a point considerably proximal to the pad on the main tentacle and directly from the tissue of the tentacle. No sign of injury is present. The appearance of this tentacle suggests the probable result of further growth of the bud shown in Fig. 3, except of course the lack of the pad at the base of the bud. Fig. 2, showing a trifid tentacle, is taken from Hargitt's paper on variation (*op. cit.*). It shows two branches arising from near the end of the main tentacle which seems to be degenerate as mentioned later. The buds here do not seem to arise from the suctorial pad of the main tentacle, which is not shown, but each bud is supplied with a pad near its tip. The knob on the tentacle under consideration, however, had more the appearance of a bud than a bifurcation. This was due chiefly to its small size which rather suggested that it was a very early stage in the formation of an extra tip to the tentacle.

The bud arose from a definite base which presented almost exactly the same external appearance as the normal suctorial pad. This similarity consisted not only in the smooth appearance, due to the absence of the ectodermal ridges found on the other parts of the tentacle, but also in its concave form, and the further presence of a bend or "knee" in the tentacle at this point; all of which are characteristic of the normal suctorial pad (Figs. 3–5). The bud arose from a depression in the base (Fig. 4) due to the cup-like shape of the pad already mentioned.

No external sign of injury was found either in this pad or in the surrounding tissue. The pad was of course not functional as an adhesive organ, another functional one being present nearer the distal end of the tentacle (Figs. 3 and 5). Whether this new pad formed after the beginning of the development of the bud from the old pad, whose functional activity would thus be destroyed; or whether a second pad formed first, and a bud began to develop from the old one (which would not then be necessary) simply as a result of the capacity for regeneration, or rather duplication of parts, inherent in the tentacles, is an extremely interesting question. Of course no direct answer can be

made without a considerable body of facts before us, coming from actual observations on this particular point; facts which it would be very difficult if not impossible to obtain. It seems to me, however, that we can suggest a probable answer from conditions observed in other cases, which bear more or less directly on this point. A comparison of Figs. 1 and 2 with some of those in Hargitt's paper show that buds do not always arise from pads. Indeed of the six figures of bifid and trifid tentacles shown in that paper and in this, only two show the bud as arising from the pad. This alone would show quite conclusively that there is no necessary or regular connection between the bud

FIG. 3.

FIG. 4.

FIG. 5.

and the suctorial pad. This is further emphasized by the fact that I found nine tentacles having each three pads, apparently well developed and functional, and yet there was not the least sign of buds arising from any of the pads. These facts all point directly toward an unusual predisposition to a duplication of organs, and this perhaps offers the most satisfactory explanation of the budding and bifurcation of tentacles.

The bud showed a constriction near its middle region, but did not present externally any signs of annulation, or rather of the ectodermal ridges present on the old tentacle. However, nematocysts were present in abundance. The general appearance of the

bud is similar to that of the old tentacle, with the exception noted above, and is undoubtedly of the same structure.

Hargitt (*op. cit.*, p. 244) in referring to bifid and trifid tentacles suggested that they might have orginataed is the result of some injury to the distal end of the tentacle. This seemed to be especially indicated by the one trifid tentacle found. In this case there seemed to be a degeneration or atrophy of the median branch, which was probably the end of the original tentacle. From the sides of this tentacle two branches arose opposite each other which were considerably longer than the median tip (*cf.* Fig. 2). He says concerning the cause of this: "The degenerating middle tip would very naturally suggest the probability that an injury might have been the predisposing cause of the secondary tips; on the other hand, it must not be overlooked that in each of the other specimens with double tips no such cause seems at all evident."

It was with the thought of trying to determine whether there was any sign of injury which might have influenced the formation of a bud in that region, as well as to determine the histogenic changes involved in its formation, that I was led to undertake a careful study of this budding tentacle.

The tentacle was stained in toto with borax carmine. Sections were cut transversely across the tentacle, thus making the sections of the bud longitudinal.

Fig. 6 represents a section of the entire tentacle showing the bud in its general relations. The entoderm of the bud is seen to be directly continuous with the entoderm of the tentacle. The bud is solid with the exception of a cavity at the distal end and there is no connection between this cavity and the cavity of the tentacle. It will be noticed, however, that the cells are arranged more or less definitely in two rows with the dividing line quite distinctly marked in the proximal region, as though in further growth these would pull apart and thus connect the distal cavity of the bud with the cavity of the tentacle. On either side of the bud are masses of the rather dense tissue which makes up the suctorial or adhesive pad already mentioned. This tissue resembles very much muscular tissue rather than glandular tissue, suggesting that the pad acts by virtue of its muscularity, rather than by

means of a secreted adhesive substance as suggested by Perkins.[1] At the edges of this pad is present the collar-like expansion of the ectoderm which is characteristic of this structure normally. Indeed, the shape, size and contents of these cells, as well as their method of staining and general appearance, is almost exactly the same as in the normal pad. Thus there seems little doubt that there was originally present here a functional suctorial pad. So

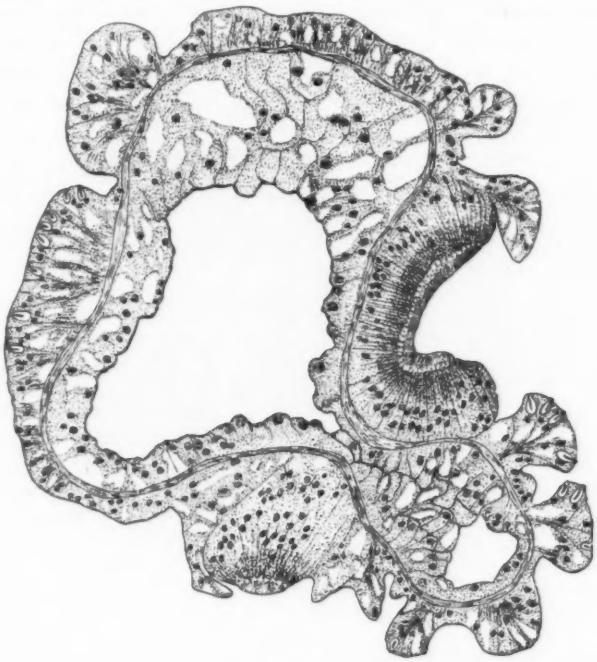


FIG. 6.

far as we can tell from the sections the pad might have formed after the bud began its development, though this is very unlikely, since in such case the function would be quite limited. Indeed the pad would lose its power of functioning normally if the bud increased in size to any extent. The ectoderm of the bud is thrown into the folds or ridges, which are characteristic of the normal tentacles, and nematocysts are limited to these ridges (*cf.* figures).

[1] "The Development of *Gonionema Murbachii*," *Proc. Acad. Nat. Sci.*, Philadelphia, p. 764, 1902.

Figs. 7, 8, 9 show only the bud and the tissues immediately adjoining. The same general features already noted are also seen here. In Fig. 7 the muscular pad on each side of the base of the bud is nearer the same size than in Fig. 6. The relation of the entoderm of the bud and tentacle is shown in about the same way, but the arrangement of the bud entoderm into two rows is not so distinctly marked. In Fig. 8 this arrangement of entoderm is scarcely indicated, the cells being more or less massed together and not showing any apparent regularity. The character of the tissue of the muscular pad is shown better as are also the ectodermal ridges. The cavity at the distal end of the bud

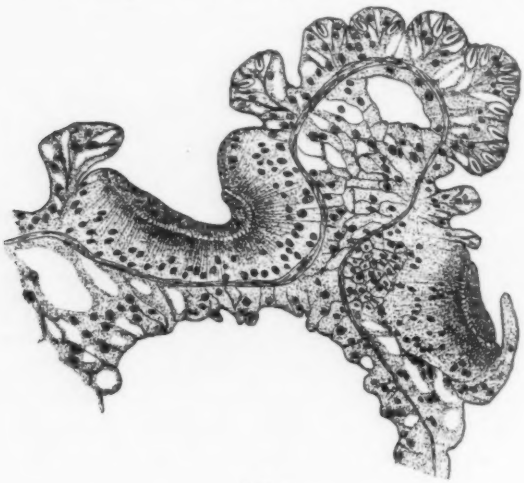


FIG. 7.

is larger than shown in the other figures. In Figs. 8 and 9 the muscular pad is shown on only one side of the base of the bud, showing that the bud is not completely surrounded by it, a feature also indicated in Figs. 3 and 5, where the pad is seen to have a notch or sinus on one side. Fig. 9 represents a section cut one side of the long axis of the bud, so that the entoderm of the bud and tentacle is not continuous.

It will have been noticed that in all the figures the bud is solid with the exception of a cavity at the distal end. Perkins (*op. cit.*, p. 785) referring to the development of the normal tentacle states

that it is at first solid, but that later "the cavity of the circular canal is drawn into it." "The entodermal cells, arranged radially about the central axis, thicken until they are forced away from the center and a tubular cavity is left." This process, he states, begins at the proximal end and the cavity is gradually "carried out along the axis of the tentacle toward the tip." In this bud there seems to be present the cavity at the distal end so

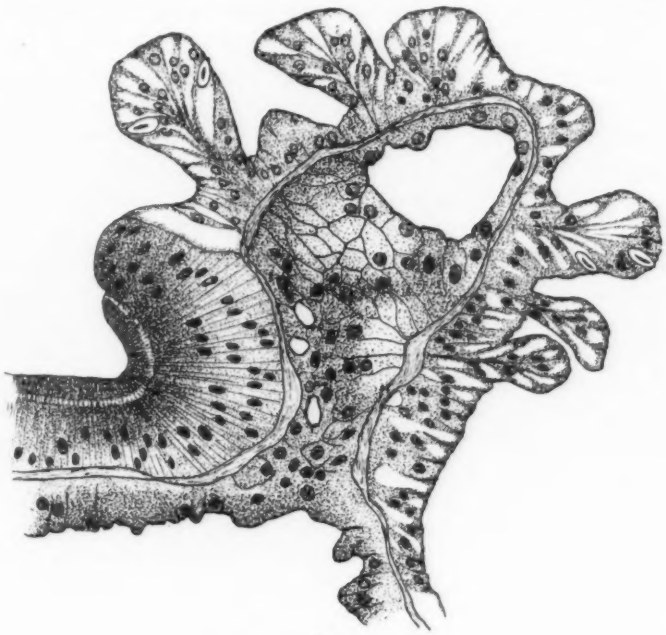


FIG. 8.

that the method just mentioned does not apply, at least not wholly. In Fig. 6 the cells are evidently arranging themselves in rows next the supporting layer, with their edges meeting near the center, suggesting a drawing away from the axis and a formation of a cavity (as Perkins suggests) connecting the cavity of the tentacle with the cavity already formed in the bud. Fig. 9, however, would seem to indicate a somewhat modified process. The cell outlines are not distinct, so that their arrangement cannot be definitely determined, but in the central part of the core are a

number of irregular cavities. This suggests the possibility of these cavities enlarging and running together, the cells at the same time taking up a regular position next the supporting layer, and thus the cavity of the bud being formed. In neither case, however, would the process necessarily begin at the proximal end. Furthermore, it is not quite certain just how the cavity at the distal end of the bud forms, or why it should form so early and not involve the proximal portion of the bud.

In regard to the early method of formation of the bud little can be suggested since it has developed beyond the initial stage. It can be said, however, that not the slightest trace of injury was found, which might be a predisposing cause. Alb. Lang[1] from

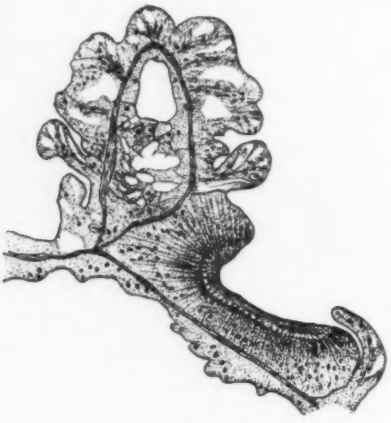


FIG. 9.

his work on budding in *Hydra*, *Eudendrium* and *Plumularia* tried to show that the bud originated from the ectoderm entirely, that the previous view that both layers were active could not be maintained ; ectoderm cells migrated through the supporting layer and formed the bud entoderm, the old entoderm being absorbed. Seeliger[2] and Braem,[3] however, working on *Hydra*, *Eudendrium*, *Plumularia*, *Obelia* and *Sertularella* claimed that Lang's results were entirely incorrect and the conclusions drawn from them

[1] *Zeitschr. f. wiss. Zool.*, Bd. 54, 1892, pp. 365–385.
[2] *Zeitschr. f. wiss. Zool.*, Bd. 58, 1894, pp. 152–188.
[3] *Biol. Centralbl.*, Bd. 14, 1894, pp. 140–161.

not warranted. They found dividing cells in the entoderm as well as in the ectoderm, a condition which Lang did not find. They found no trace of ectoderm cells migrating into the entoderm even in the earliest stages, of the two layers running into each other, of the entoderm being pushed aside and absorbed. Many others also have questioned Lang's claims and maintain that results obtained in many species of hydroids, as well as in other forms of animal life, confirm the old view. My own work on hydroids [1] confirm these results of Seeliger, Braem and others. In working on the regeneration of *Tubularia crocea*, *T. tenella*, *T. larynx*, *Eudendrium ramosum* and *Pennaria tiarella* particular attention was paid to structures which form either wholly or in part by budding. Sections made through these buds in all stages showed none of the features claimed by Lang, such as migrating entoderm cells, dissolution of the supporting layer with the accompanying disappearance of sharp contrast between the two layers, and absorption of the entoderm. Often, usually indeed, the entoderm seemed to be the layer most active in this process. Mitotic division was observed in both ectoderm and entoderm though not abundant, reasons for which are discussed in the above-mentioned paper. Amitotic division was also more or less prevalent in both layers. Perkins (*op. cit.*, p. 784) referring to the formation of the tentacle in *Gonionemus* says the three layers, ectoderm, entoderm and supporting layer "are pushed out somewhat in the growth of the tentacle, the region of greatest activity being the endodermal layer, where the core of the tentacle is formed by a rapid outgrowth of the cells of the body wall accompanied by multiplication of these same cells." He further states that there is not even an initial thickening of the ectoderm in the region where the tentacle is to appear. In regard to budding in the larval form likewise, he states that the ectoderm and entoderm cells divide, the entoderm pushes out gradually, the ectoderm growing so regularly as to cover it with a layer of constant thickness. Thus it has been conclusively proved that budding in many hydroids, as well as in the formation of the tentacle and in other buds of *Gonionemus*, involves both layers, and cells in both layers increase rapidly by division. This would entirely dis-

[1] *Arch. f. Ent-mech.*, Bd., XVII., Heft 1, 1903, pp. 64–91.

credit the universality of Lang's claims, even if they held true for a few forms.

Since this is the method of the formation of the tentacles, and of buds in other regions of *Gonionemus*, we may safely assume that probably the same process would be active in the formation of the bud from the tentacle, or at least to a great extent. Greater support is given this assumption by the fact of the presence of mitotically dividing nuclei found in the entoderm of the bud. They were not definitely determined in the ectoderm though doubtless present.

I am glad to acknowledge my indebtedness to my father, C. W. Hargitt, for permission to reproduce Fig. 2, which appeared in his paper on "Variation among Hydromedusæ," and also for allowing me to examine his collections of *Gonionemus*.

ZOÖLOGICAL LABORATORY, SYRACUSE UNIVERSITY,
January 18, 1904.